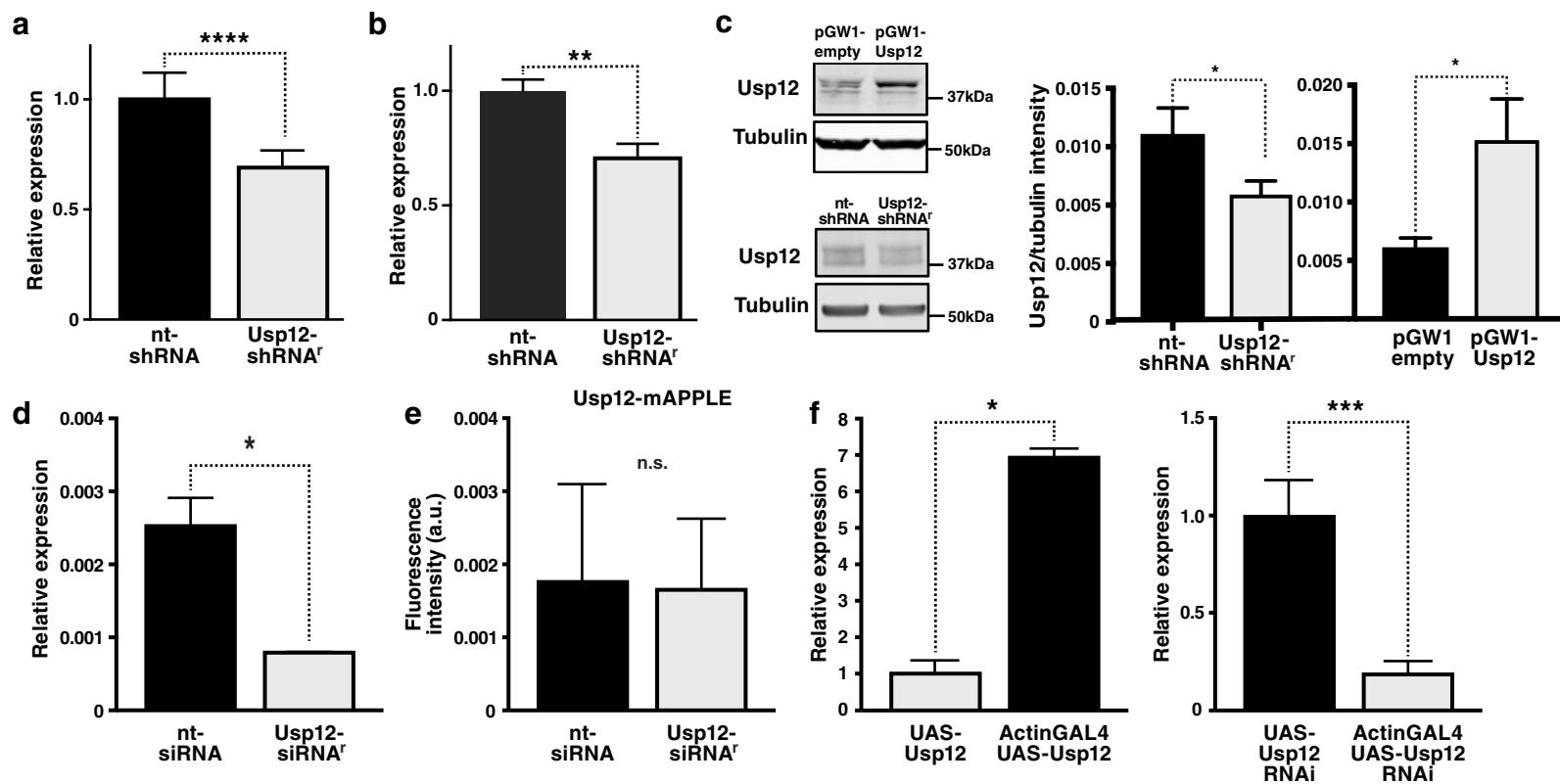


Deubiquitinase Usp12 functions noncatalytically to induce neuronal autophagy and confer neuroprotection in models of Huntington's disease

Aron et al.

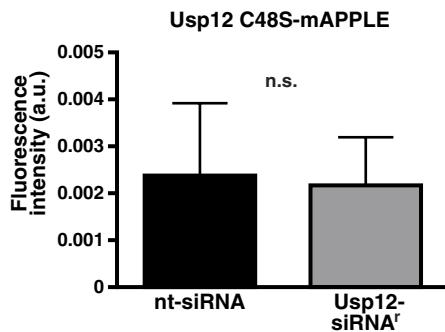
Supplementary Figure 1



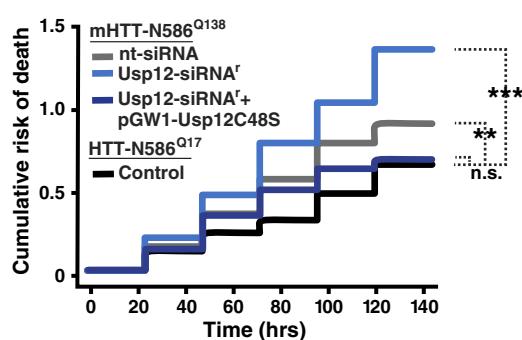
Supplementary Figure 1. shRNA-mediated knockdown of Usp12 in PC12 cells and primary neurons. (a) qPCR analysis of Usp12 mRNA levels in rat cell line (PC12) transfected with Usp12-shRNA^r plasmid. With approximately 50% transfection efficiency, as measured by GFP plasmid co-transfection, Usp12 mRNA knockdown averaged 30–50%. Atp5b is used as the reference gene for quantification. Usp12 mRNA levels in Usp12-shRNA^r treated cells are then normalized to Usp12-mRNA levels in cells treated with nt-shRNA. (b) qPCR analysis of Usp12 mRNA levels in primary rodent neurons transduced with Usp12-shRNA^r lentivirus. Due to the low transfection efficiency of primary neurons (<1–5%), we used a lentivirus-mediated shRNA delivery approach to confirm the effectiveness of Usp12-shRNA^r in primary neurons. Primary rat neurons transduced with Usp12-shRNA^r lentivirus show ~30% reduction in Usp12 mRNA levels. (c) Western blot analysis of Usp12 levels of a rat C6 cell line transfected with either the shRNA or the overexpression vector pGW1-Usp12. Graph bars on the right represent the relative intensity of Usp12. (d) Relative Usp12 mRNA levels of a rat C6 cell line transfected with Usp12-siRNA^r (e) Fluorescence intensity of the pGW1-Usp12-mAPPLE plasmid co-transfected with the Usp12-siRNA^r or non-targeting siRNA ($p=0.66$). (f) Relative mRNA levels of Usp12 overexpression and RNAi knock-down in control fruit flies, driven with the actin-GAL4 driver (see Methods). * $p<0.01$ ** $p<0.001$, *** $p<0.0001$. Error bars represent s.e.m.

Supplementary Figure 2

a

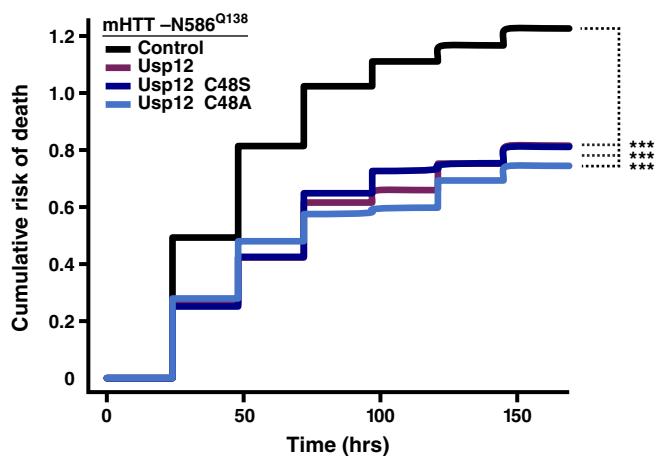


b



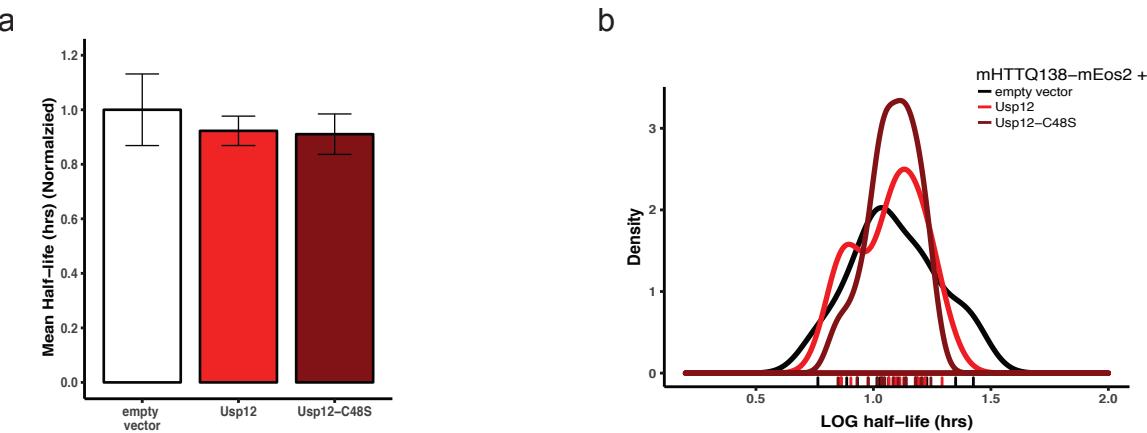
Supplementary Figure 2. The catalytic dead Usp12-C48S rescues the Usp12-siRNA^r mediated toxicity. (a) Fluorescence intensity of the pGW1-Usp12-C48S-mAPPLE plasmid co-transfected with the Usp12 siRNA^r or non-targeting siRNA ($p=0.29$). (b) Cumulative risk of death of the mHttQ138 when co-transfected with either control siRNA, Usp12-siRNA^r or Usp12-siRNA^r + pGW1-Usp12(C48S) * $p<0.01$ ** $p<0.001$, *** $p<0.0001$, n.s.=not significant. Statistical information is summarized in Table 3.

Supplementary Figure 3



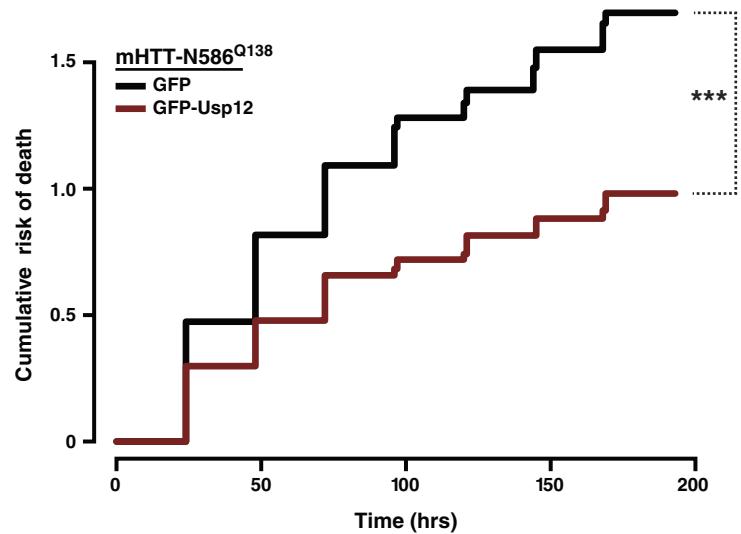
Supplementary Figure 3. The active-site cysteine mutant Usp12-C48A suppresses mHTT-N586Q138 toxicity similar to Usp12-C48S. Cumulative risk of death plot for primary neurons co-transfected with mHTT-N568Q138 and empty vector, Usp12, Usp12-C48S, or Usp12-C48A. mHTT-N568Q138 + empty vector vs. Usp12: HR=0.6, 95% CI (0.4750– 0.8134), p=0.000531; vs. Usp12-C48S: HR=0.6, 95% CI (0.4704–0.8222), p=0.000854; vs. Usp12-C48A: HR=0.6, 95% CI (0.4446–0.7788), p=0.000208). Number of neurons per group: mHTT-N568Q138 + empty vector, n=167; mHtt-N568Q138 + Usp12, n=174; mHTT-N568Q138 + Usp12-C48S, n=153; mHtt-N568Q138 + Usp12-C48A, n=160. ***p<0.001.

Supplementary Figure 4



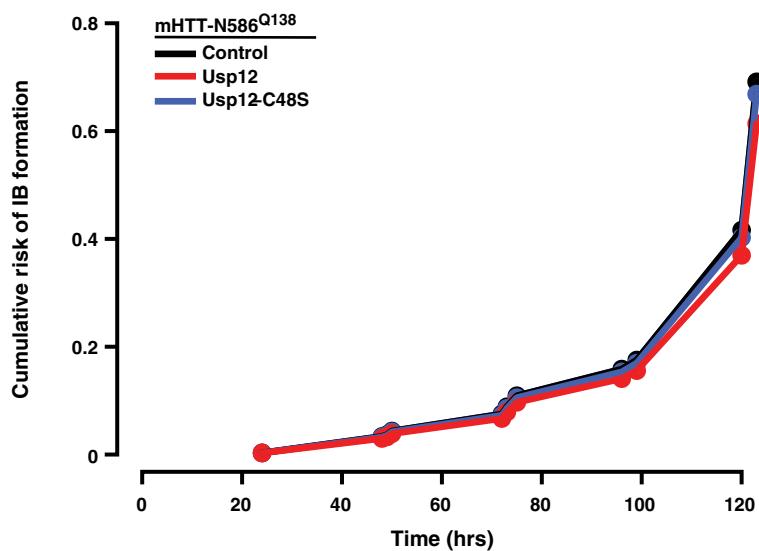
Supplementary Figure 4. Usp12 overexpression does not significantly affect the half-life of soluble mHTT in rat primary cortical neurons. Optical pulse-labeling was used to measure the effect of Usp12 or Usp12-C48S on half-life of mHTT-N586Q138-mEos2, similar to the assay described for LC3 half-life (Fig. 6). To measure the effect on only soluble/diffuse mHTT protein, neurons with visible inclusion bodies were excluded from the analysis. (a) Mean half-life of photoconverted mHTT-N586Q138-mEos2 in neurons co-expressing either empty vector, Usp12, or Usp12-C48S. (b) Half-life distributions of individual neurons from (a). Number of neurons per group for representative experiments (n): mHTT-N586Q138+empty vector, n=16; mHTT-N586Q138+Usp12, n=20, mHTT-N586Q138+Usp12-C48S, n=20. Error bars represent s.e.m. Statistical significance of means and distribution determined by Mann-Whitney and Kolmogorov-Smirnov tests, respectively.

Supplementary Figure 5



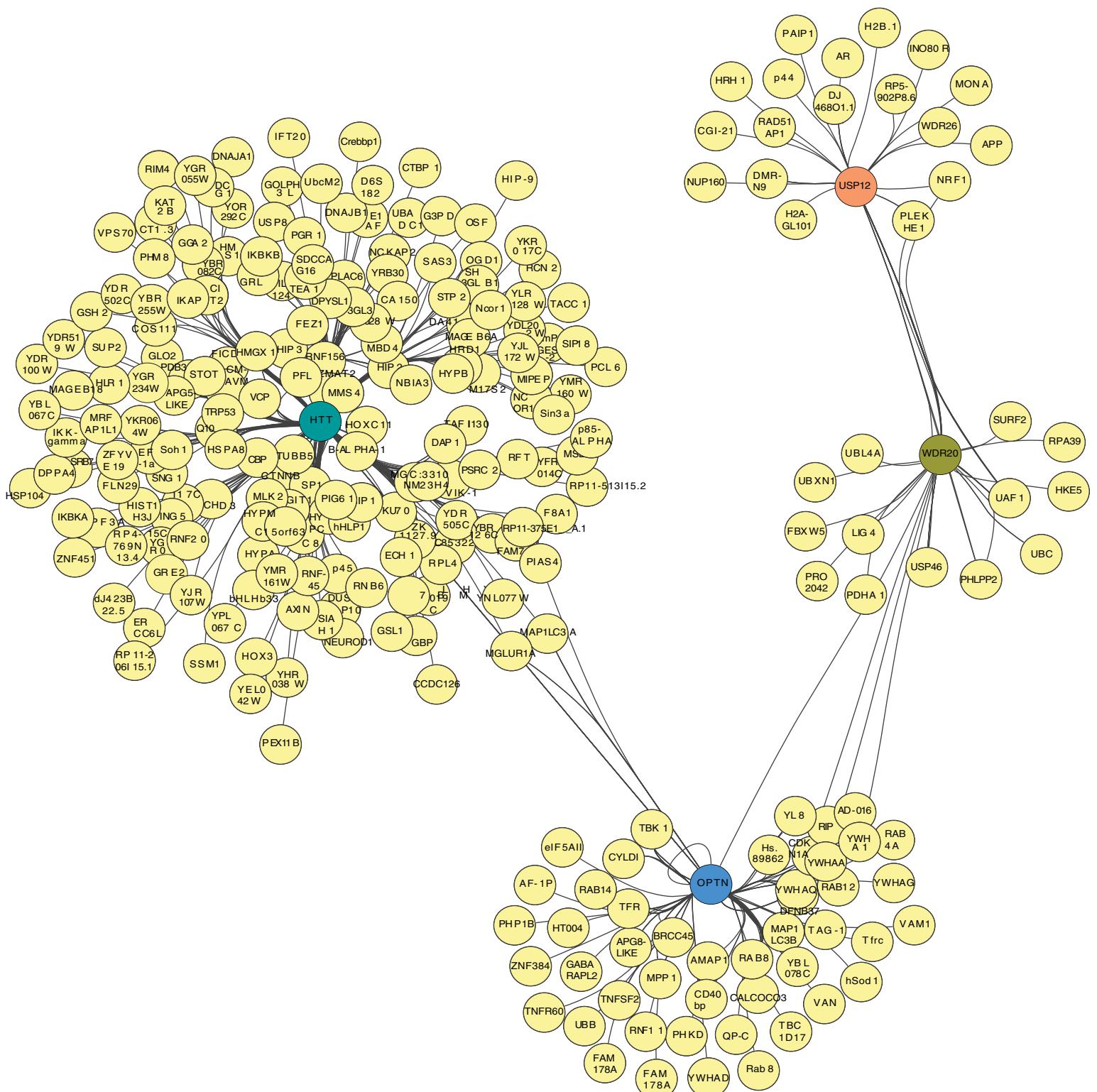
Supplementary Figure 5. Fluorescent protein-tagged Usp12 suppresses mHTT toxicity similar to untagged Usp12. Cumulative risk of death plot for primary neurons co-transfected with mHTT-N568Q138 and empty vector or GFP-Usp12. Two experiments combined. Relative risk of death: mHTT-N568Q138 + empty vector vs. mHTT-N568Q138 + GFP-Usp12, HR=0.6, 95% CI (0.494–0.6936), $p=5.85\text{e-}10$. Number of neurons per group: mHTT-N568Q138 + empty vector, $n=381$, mHTT-N568Q138 + GFP-Usp12, $n=384$. *** $p<0.001$

Supplementary Figure 6



Supplementary Figure 6. Cumulative risk of inclusion body formation in neurons expressing mHTT-N586-Q138 and either empty vector, Usp12, or Usp12-C48S. Results are from three experiments combined. Number of neurons per group: mHtt-N568Q138 + empty vector, n=376, mHTT-N568Q138 + Usp12, n=283, mHtt-N568Q138 + Usp12-C48S, n=332.

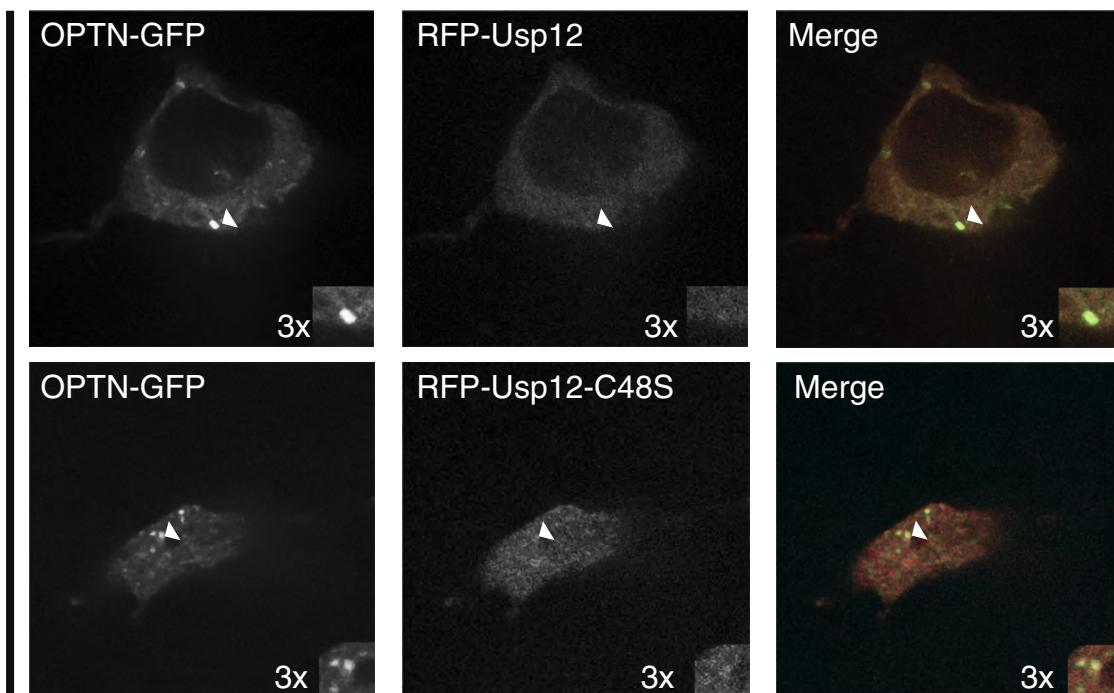
Supplementary Figure 7



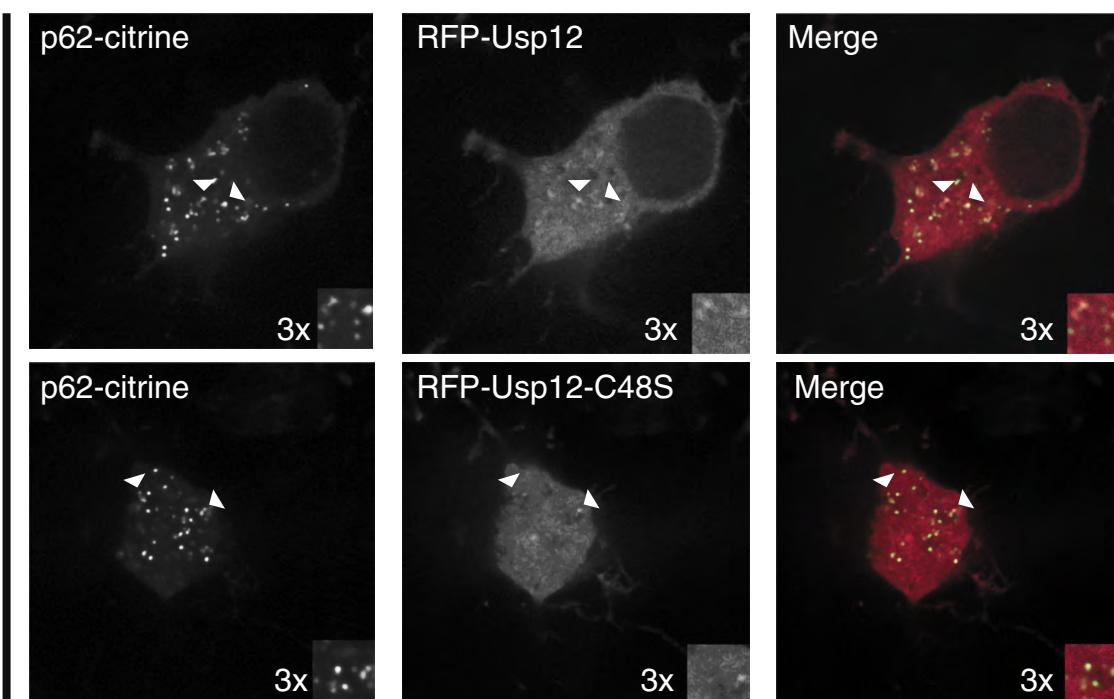
Supplementary Figure 7. Detailed map of experimentally validated protein-protein interactions linking Usp12 with the autophagy receptors Optineurin (Optn) and mHTT, based on data collection in BioGRID. Further information is available at www.thebiogrid.com.

Supplementary Figure 8

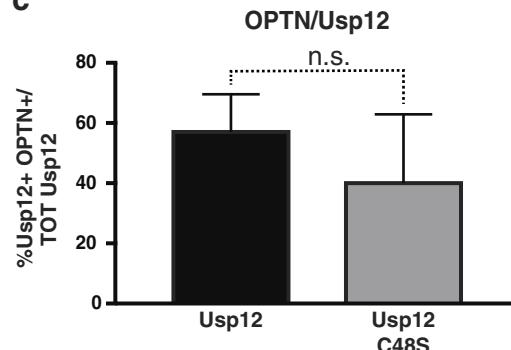
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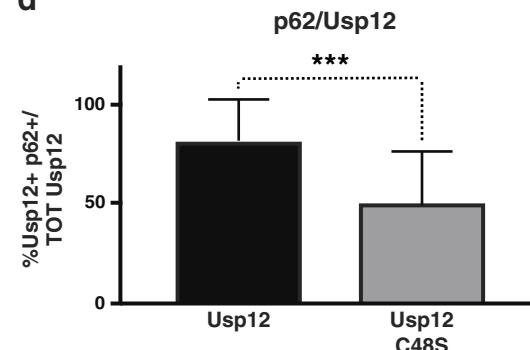
b



c



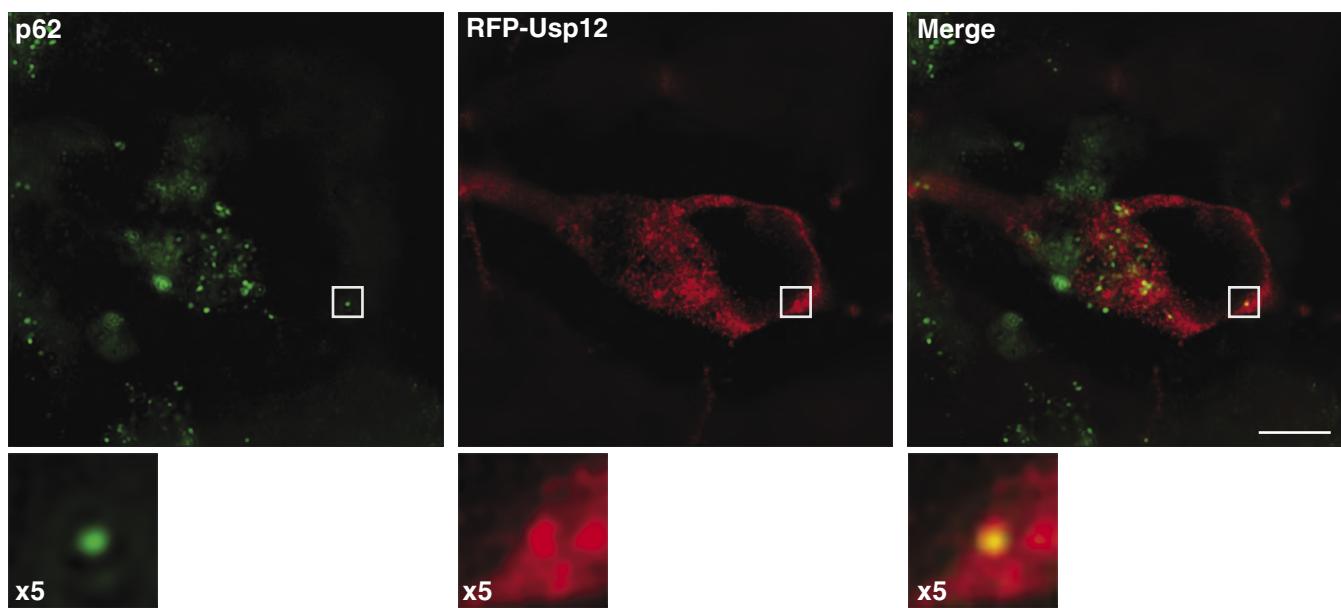
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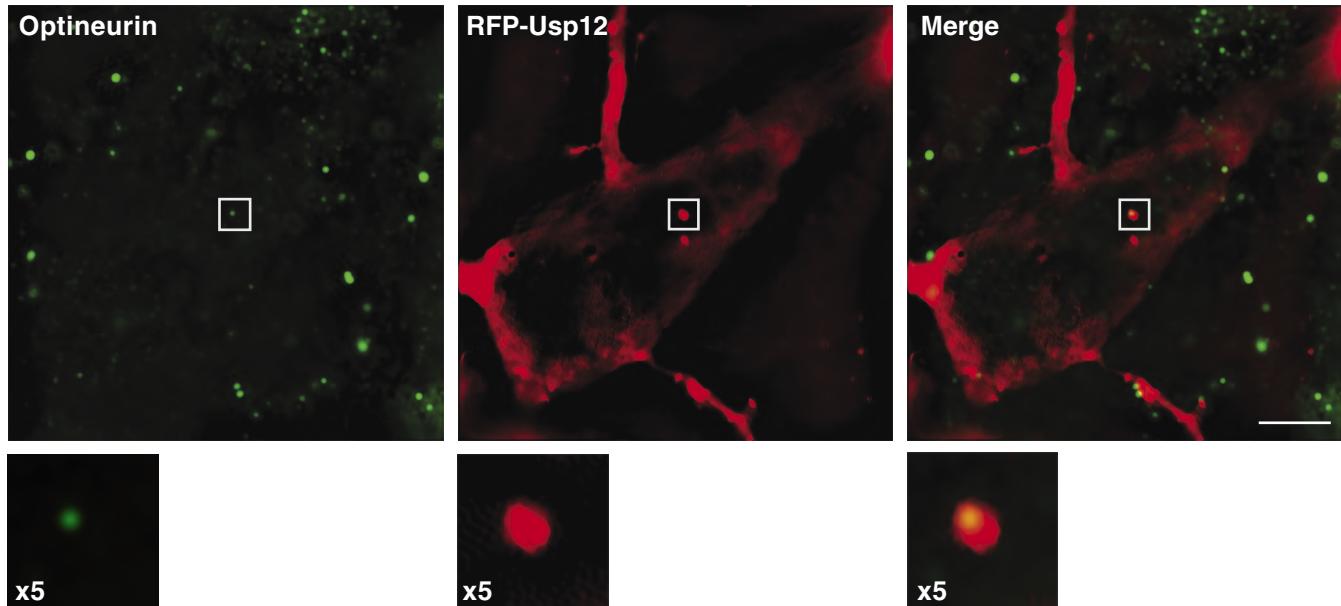
Supplementary Figure 8. Usp12 localizes to autophagy receptors. (a,b) Representative images of RFP-Usp12 co-transfected with either Optineurin (Optn)-GFP (a) or p62-citrine (b) = in primary neurons. Images were captured in fixed cells approximately 48 h after transfection and after 3 h incubation with Bafilomycin A. Scale bar=10 μ m. (c-d) quantification of the Usp12 puncta that co-localize with either OPTN or p62, at least 10-15 cells per condition were quantified. Student's t-test was used to compare groups * $p<0.01$ *** $p<0.0001$, n.s.=not significant. Data are representative of three independent experiments. Error bars represent s.e.m..

Supplementary Figure 9

a

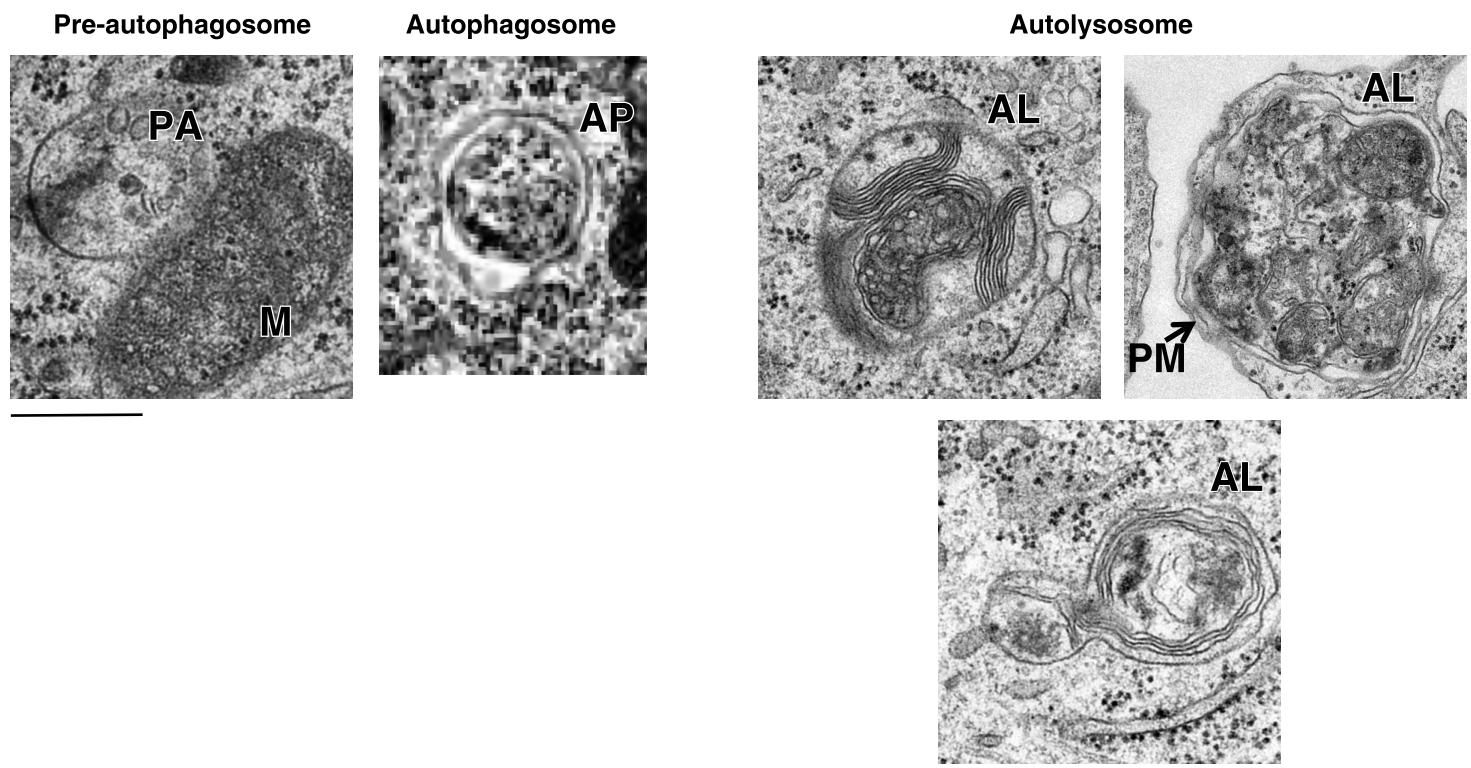


b



Supplementary Figure 9. Representative examples of reconstructed super-resolution images are shown for each pattern. Usp12-mAPPLE expressing cells were incubated with anti-p62 antibody (a) or anti-optineurin antibody (b). Colocalized puncta are represented in the insets. Scale bar: 5 μ m

Supplementary Figure 10



Supplementary Figure 10. Examples of autophagic structures included in analysis. PA=pre-autophagosome, M=mitochondria, AP=autophagosome, AL=autolysosome, PM=plasma membrane. Scale bar= 500 nm

Supplementary Figure 11

Figure 3g

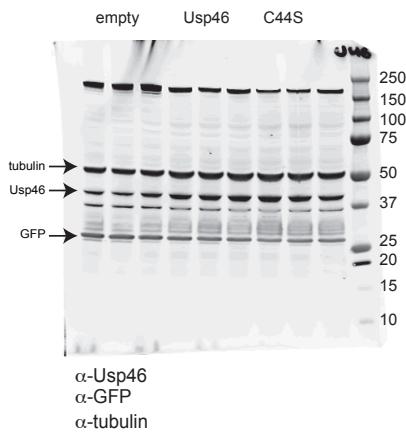


Figure 4d

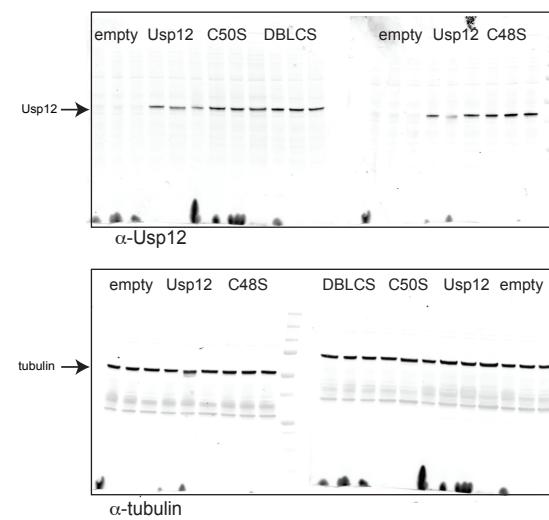


Figure 5e

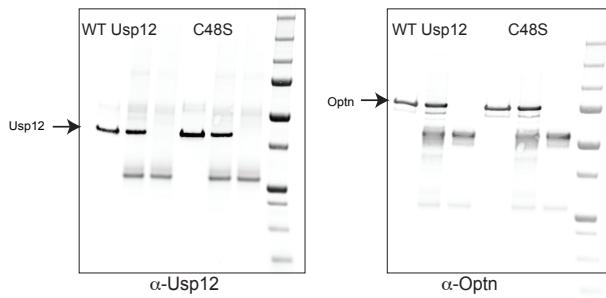
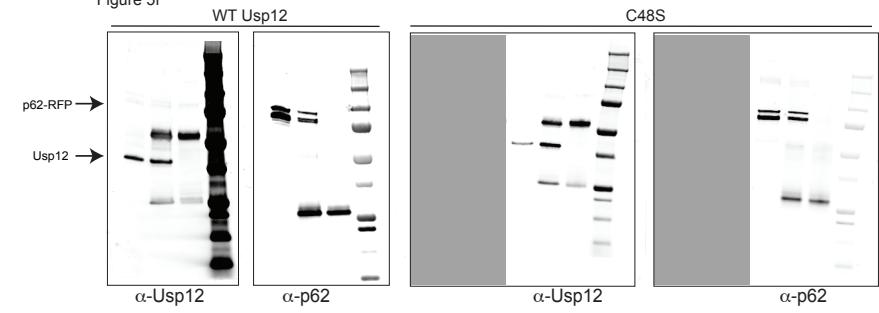
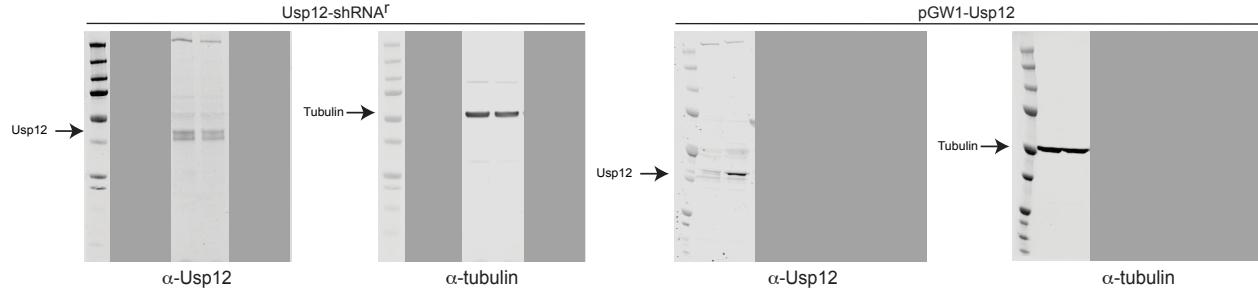


Figure 5f



Supplementary Figure 1c



■ Data not used in this manuscript

Supplementary Figure 11. Full immunoblots. Molecular mass marker labeled in full blot for Figure 3f, and is the same marker in all other blots.

Supplementary Table 1. Cox proportional hazards analysis of the effects of Usp12 knockdown on survival of rodent primary neurons

	HR	HR 95% CI	p value	n
HTT-N586 Q17	—	—	—	258
nt-siRNA				
Usp12-siRNA ^r	1.15	0.85–1.56	0.34	506

HR, hazard ratio; n, number of neurons; CI, confidence interval

Supplementary Table 2. Cox proportional hazards analysis of the effects of Usp12 knockdown or overexpression on survival of rodent primary neurons.

	HR	95% CI	p value	n
HTT-N586 ^{Q17}	—	—	—	240
mHTT-N586 ^{Q138}				
nt-siRNA + control vector	1.45	1.12–1.89	0.0054	196
Usp12-siRNA ^r + control vector	2.10	1.67–2.66	6.2e-10	260
Usp12-siRNA ^r + Usp12	1.33	1.04–1.71	0.0241	238
Usp12-siRNA ^r + Usp12 + C48S	1.14	0.84–1.54	0.3961	136

HR, hazard ratio; n, number of neurons; CI, confidence interval

Supplementary Table 3. Cox proportional hazards analysis of Usp12 effect on survival of patient-derived iPSC-differentiated neurons.

	HR	HR 95% CI	p value	n
Control + Control vector	Reference	—	—	129
Control + Usp12	0.97	0.62–1.50	0.8775	128
HDQ109 + Control vector	1.49	1.02–2.19	0.0394	179
HDQ109 + Usp12	0.94	0.63–1.41	0.7759	201
Control + Control vector	0.67	0.46–0.98	0.03942	129
Control + Usp12	0.65	0.44–0.95	0.02774	128
HDQ109 + Control vector	Reference	—	—	179
HDQ109 + Usp12	0.63	0.45–0.89	0.00856	201

HR, hazard ratio; n, number of neurons; CI, confidence interval

Supplementary Table 4. Cox proportional hazards analysis of Usp12 effect on survival of neurons expressing toxic neurodegenerative disease-related proteins

Usp12 overexpression (ALS model)	HR	HR 95% CI	p value	n
GFP+ Control vector	Reference	—	—	194
GFP+ Usp12	1.04	0.80–1.35	0.779	239
TDP-43 + Control vector	1.90	1.48–2.42	3.30e-07	235
TDP-43+ Usp12	2.27	1.78–2.90	3.04e-11	243
GFP + Control vector	Reference	—	—	151
GFP + Usp12	0.89	0.65–1.23	0.482	150
TDP-43 ^{A315T} + Control vector	1.97	1.49–2.60	1.81e-06	168
TDP-43 ^{A315T} + Usp12	2.39	1.82–3.13	3.75e-10	186
Usp12 overexpression (PD model)				
GFP + Control vector	Reference	—	—	488
GFP + Usp12	1.07	0.90–1.27	0.417	446
α -synuclein + Control vector	1.44	1.24–1.69	3.45e-06	534
α -synuclein + Usp12	1.63	1.39–1.90	1.41e-09	483

HR, hazard ratio; n, number of neurons; CI, confidence interval

Supplementary Table 5. Cox proportional hazards analysis of differential effects of Usp46 on neuron survival

Usp46 overexpression	HR	HR 95% CI	p value	n
HTT-N586 ^{Q17} + Control vector	Reference	—	—	382
HTT-N586 ^{Q17} + Usp46	1.78	1.46–2.17	1.67e–08	394
mHTT-N586 ^{Q138} + Control vector	1.85	1.52–2.25	1.10e–09	411
mHTT-N586 ^{Q138} + Usp46	1.83	1.50–2.23	2.89e–09	386
WT and mutant Usp46 overexpression				
HTT-N586 ^{Q17} + Control vector	Reference	—	—	160
HTT-N586 ^{Q17} + Usp46	2.25	1.63–3.11	7.76e–07	146
HTT-N586 ^{Q17} + Usp46-C44S	1.26	0.83–1.92	0.276	74
mHTT-N586 ^{Q138} + Control vector	Reference	—	—	192
mHTT-N586 ^{Q138} + Usp46	0.98	0.74–1.31	0.912	137
mHTT-N586 ^{Q138} + Usp46-C44S	0.97	0.73–1.28	0.832	149
WT and mutant Usp12/Usp46 overexpression				
GFP + Control vector	Reference	—	—	178
GFP + Usp46	1.84	1.40–2.43	1.6e–05	173
GFP + Usp46-C44S	1.01	0.74–1.37	0.947	165
GFP + Usp12	0.97	0.71–1.33	0.857	158
GFP + Usp12-C48S	1.18	0.87–1.64	0.317	119

HR, hazard ratio; n, number of neurons; CI, confidence interval

Supplementary Table 6. Cox proportional hazards analysis of the effects of Wdr20 and Wdr48 knockdown in survival of mHTT neurons

	HR	95% CI	p value	n
mHTT-N586 ^{Q138}				
Control vector + nt-siRNA	Reference	—	—	551
Usp12-C48S + nt-siRNA	0.69	0.59–0.81	4.52e–06	292
Usp12-C48S + Wdr48-siRNA	0.65	0.56–0.75	6.28e–09	406
Usp12-C48S + Wdr20-siRNA	0.67	0.58–0.78	7.72e–08	391
Usp12-C48S + Wdr48-siRNA + Wdr20-siRNA	0.59	0.50–0.69	5.84e–09	280

HR, hazard ratio; n, number of neurons; CI, confidence interval.

Supplementary Table 7. Effect of Usp12 on mHTT-N586^{Q138}-related risk factors, expression level and IB formation, on neuronal survival.

mHTT-N586 ^{Q138} +	Covariate	HR	95% CI	p value
Empty vector (n=376)	mHTT-N586 ^{Q138} fluorescence (a.u.)	6.30776	2.802–14.200	8.7e–06 ***
	IB formation	0.15909	0.052–0.487	0.00127 **
	IB formation:time	1.03065	1.013–1.048	0.00043 ***
Usp12-WT (n=293)	mHTT-N586 ^{Q138} fluorescence (a.u.)	1.4579	1.2888–1.649	2.1e–09 ***
	IB formation	0.0932	0.0166–0.522	0.0069 **
	IB formation:time	1.0324	1.0093–1.056	0.0057 **
Usp12-C48S (n=332)	mHTT-N586 ^{Q138} fluorescence (a.u.)	8.95395	4.9181–16.302	7.5e–13 ***
	IB formation	0.14141	0.0401–0.499	0.0024 **
	IB formation:time	1.02791	1.0094–1.047	0.0030 **

IB, inclusion body; n, number of neurons; HR, hazard ratio; CI, confidence interval; a.u., arbitrary unit.

Supplementary Table 8. Competing risks regression analysis of the effects of Usp12 on risk of mHTT-N586^{Q138}IB formation.

mHTT-N586 ^{Q138} +	HR	95% CI	p value
Empty vector (n=376)	Reference	—	—
Usp12-WT (n=293)	0.918	0.714–1.18	0.58
Usp12-C48S (n=332)	1.034	0.801–1.33	0.83

IB, inclusion body; n, number of neurons; HR, hazard ratio;

CI, confidence interval

Supplementary Table 9. Cox proportional hazards analysis of the effects of Usp12 on mHTT toxicity during autophagy inhibition by Atg7 knockdown

		HR	95% CI	p value	n
mHTT-N586 ^{Q138} +					
nt-shRNA	Control	Reference	—	—	504
	Usp12	0.67	0.537–0.827	2.4e–04	411
	Usp12-C48S	0.52	0.402–0.675	7.7e–07	302
Atg7-shRNA	Control	Reference	—	—	406
	Usp12	1.01	0.847–1.20	0.92	446
	Usp12-C48S	0.97	0.810–1.16	0.73	415

HR, hazard ratio; n, number of neurons; CI, confidence interval.